

A chromosomal analysis of three species of *Timarcha* (Coleoptera, Chrysomelidae, Chrysomelinae)

Eduard Petitpierre¹

¹ Dept. Biologia, Universitat de les Illes Balears, 07122 Palma de Mallorca, Spain

Corresponding author: Eduard Petitpierre (eduard.petitpierre@uib.es)

Academic editor: N. Golub | Received 3 July 2015 | Accepted 25 September 2015 | Published 22 January 2016

<http://zoobank.org/85651D56-196D-4EF6-BF3A-AB7EB987F552>

Citation: Petitpierre E (2016) A chromosomal analysis of three species of *Timarcha* (Coleoptera, Chrysomelidae, Chrysomelinae). Comparative Cytogenetics 10(1): 27–32. doi: 10.3897/CompCytogen.v10i1.5570

Abstract

The karyotypes of three species of *Timarcha* Latreille, 1829 have been analysed. *T. (Metallotimarcha) metallica* (Laicharting, 1781), has $18 + X_y$ male meioformula and $2n = 38$ chromosomes, similar to those found in the two species of subgenus *Americanotimarcha* Jolivet, 1948, in agreement with morphological and molecular phylogenetic grounds. *T. (Timarcha) carmelena* Petitpierre, 2013 displays $9 + X_y$ and $2n = 20$ chromosomes as in morphologically related Andalusian species, whereas *T. (Timarcha) parvicollis* ssp. *seidlitzii* Kraatz, 1879 shows $11 + X_{yp}$ and $2n = 24$ chromosomes, clearly differing from the previous species. These results are discussed in order to get an insight into the main trends of the chromosomal evolution in *Timarcha*.

Keywords

Coleoptera, Chrysomelidae, Chrysomelinae, karyotypes, *Timarcha*, evolution

Introduction

The highly speciose genus *Timarcha* Latreille, 1829 comprises more than three hundred described taxa, almost all from the Palearctic (Gómez-Zurita 2008, Kippenberg 2010, Warchalowski 2010), and is relatively well-known from chromosomal standpoints because 42 taxa have been surveyed to date and their range of diploid numbers goes from $2n = 18$ to $2n = 44$ (Gómez-Zurita et al. 2004, Petitpierre 2011).

Herein, we report the chromosome numbers, male sex-chromosome systems, and main features of their karyotypes of *T. (Metallotimarcha) metallica* (Laicharting, 1781), *T. (Timarcha) carmelenae* Petitpierre, 2013 and *T. (Timarcha) parvicollis* ssp. *seidlitzii* Kraatz, 1879 to enlarge the cytogenetic analysis of the genus and discuss the most relevant trends of its chromosomal evolution.

Material and methods

The three checked species and their geographical origins are given in Table 1. The chromosome analyses were only performed on male living individuals brought to our laboratory in Palma de Mallorca (Spain), where they were killed with ethyl acetate. The cytogenetic data were obtained by testis dissection of male adult specimens which were fixed in 45% acetic acid , later on teased into small pieces for five minutes, squashed under a coverslip, immediately frozen in liquid nitrogen to remove the coverslip, and finally treated using conventional Giemsa staining procedures. Most examined cells were at meiotic metaphase I, providing the male meioformulae, thus the number of autosomal bivalents plus the male sex-chromosome systems. Finally, we took micrographs by a ZEISS AXIOPHOT or a ZEISS AXIOSKOP photomicroscope, and subsequently enlarged them for printing.

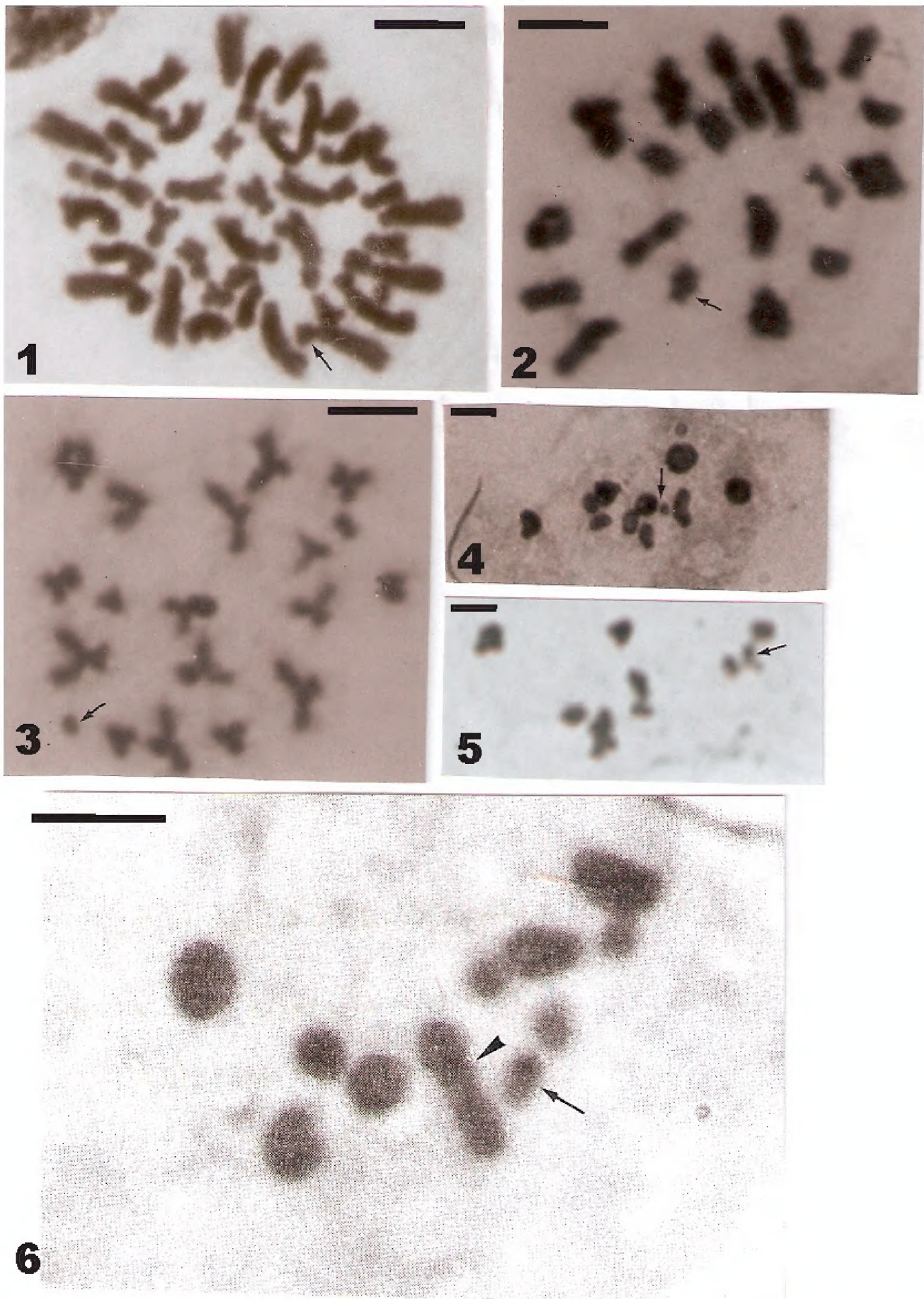
Results

Timarcha (Metallotimarcha) metallica (Laicharting, 1781)

Two males of this species have displayed 2n= 38 chromosomes and an 18 + Xy_p male meioformula, with a “parachute” Xy_p sex-chromosome system (Fig. 2). Its karyotype is composed of nine medium size and nine small autosome pairs plus a submetacentric X-chromosome of medium size and a tiny y-chromosome. Four of the medium size autosome pairs were acrocentrics and the remaining meta- or submetacentrics, and three of the small ones were acrocentrics and the other metacentrics, as shown by spermatogonial mitotic metaphases (Fig. 1) and meiotic metaphases II (Fig. 3). Thus, the fundamental number (FN) of chromosomal arms is 50.

Table 1. Chromosomally analysed species of *Timarcha* and their geographical sources. FR=France, SP=Spain.

<i>T. metallica</i> (Laicharting, 1781)	Déville: Bois de Waibes, Ardennes (FR)
<i>T. carmelenae</i> Petitpierre, 2013	P.N. Sierra de Castril: Sierra Seca, Granada (SP)
	“ La Sagra: collado de las Víboras, Granada (SP)
<i>T. parvicollis seidlitzii</i> Kraatz, 1879	Sierra Tejeda: La Maroma, Granada (SP)



Figures 1–6. 1–3 *T. metallica*: 1 spermatogonial mitotic metaphase with $2n = 38$ chromosomes, the y-chromosome is arrowed 2 meiotic metaphase I with $18 + Xy_p$ meioformula, the Xy_p is arrowed 3 meiotic metaphase II with $n = 19$ chromosomes 4–5 *T. carmelenae*: meiotic metaphases I from Sierra de Castril (4) and La Sagra (5) individuals, with $9 + Xy_p$ meioformula, the Xy_p are arrowed 6 *T. parvicollis* ssp. *seidlitzii*: meiotic metaphase I with $11 + Xy_p$ meioformula, the Xy_p is arrowed and two partly overlapped autosomal bivalents are arrowheaded. Bar: 5 μm .

***Timarcha (Timarcha) carmelenae* Petitpierre, 2013**

One male individual from Sierra Seca and another from La Sagra provided meiotic metaphases I of $9 + Xy_p$, again with a “parachute” Xy_p sex-chromosome system, that is $2n = 20(Xy_p)$ chromosomes, and showing two autosomal bivalents a bit larger than the others (Figs 4 and 5).

***Timarcha (Timarcha) parvicollis* ssp. *seidlitzii* Kraatz, 1879**

The only checked male individual provided meiotic metaphase I with an $11 + Xy_p$ meioformula, having also a “parachute” Xy_p sex-chromosome system, thus $2n = 24(Xy_p)$, where five autosomal bivalents are larger than the remaining six ones (Fig. 6).

Discussion

The diploid number of $2n = 38$ chromosomes shown in *Timarcha (Metallotimarcha) metallica* should correct a previous miscounting report of $2n = 20$ chromosomes (Petitpierre 1982). The high chromosome number found in this species is not displayed by any other *Timarcha* from the Palearctic (subgenus *Timarcha* s.str.), whose range of numbers goes from $2n = 18$ to $2n = 30$ (Gómez-Zurita et al. 2004, Petitpierre 2011). However, high chromosome numbers are characteristic of the two species of the subgenus *Americanotimarcha* Jolivet, 1948, e.i., *T. intricata* Halderman, 1854 with $2n = 44$ (Petitpierre and Jolivet 1976) and *T. cerdo* Stal, 1860 with $2n = 38$ (Jolivet and Petitpierre 1992). These high chromosome numbers are in agreement with the similar morphological traits, the male genitalia and the molecular phylogenetic resemblances between the subgenera *Metallotimarcha* Motschulsky, 1860 and *Americanotimarcha* (Jolivet 1948, Iablokoff-Khnzorian 1966, Gómez-Zurita et al. 2000, Gómez-Zurita et al. 2004, Jolivet et al. 2013). Although the species of both subgenera show some plesiomorphic features, such an incomplete fusion of elytra, weak sexual dimorphism, aedeagus with a long tegmen cap, and a basal position in the molecular phylogenetic tree, their high chromosome numbers can not be considered as an ancestral character. First, because $2n = 20(Xy_p)$ is assumed to be the plesiomorphic and most frequent karyotype condition for Coleoptera of the suborder Polyphaga (Smith and Virkki 1978, Angus et al. 2007). Besides, this is the most common karyotype in the genus *Timarcha* where more than a half of the 42 surveyed taxa show $2n = 20(Xy_p)$ (Petitpierre 2011). And third, the karyotypes of both *T. metallica* and *T. intricata* share a quite high number of acrocentric autosome pairs, seven and fourteen respectively, which is an indication of their derived origin by multiple centric fissions or chromosomal dissociations from meta- or submetacentric chromosomes. Therefore, we assume that a hypothetical karyotype of $2n = 20(Xy_p)$ chromosomes, mostly composed of metacentrics or submetacentrics, would have been the plesiomorphous state for the genus, from which all the taxa of the three present subgenera, *Americanotimarcha*, *Metallotimarcha* and *Timarcha* s.str. may have radiated.

The karyotype of *T. (T.) carmelenae* with $2n = 20(Xy_p)$, with two larger autosomal bivalents and the remaining gradually decreasing, is similar to those of *T. (T.) intermedia* Herrich-Schäffer, 1838, and *T. (T.) lugens* Rosenhauer, 1856 (Petitpierre 1970, 1976). These three species share close morphological resemblances and a feeding on Brassicaceae plants, *Hormathophylla spinosa* (L.) Küpfer, 1974 for both *T. (T.) carmelenae* and *T. (T.) lugens* (González-Megías and Gómez 2001, Petitpierre and Daccordi 2013) and *Carrichtera annua* (L.) DeCandolle, 1821 for *T. (T.) intermedia* (Petitpierre 1971, Jolivet and Petitpierre 1973), in contrast with the prevalent trophism on plants of Rubiaceae and/or Plantaginaceae reported for almost all the other taxa of the subgenus *Timarcha* s.str. (Jolivet and Petitpierre 1973).

T. (T.) parvicollis ssp. *seidlitzii* shows a karyotype of $11 + Xy_p$ male meioformula, thus $2n = 24(Xy_p)$ chromosomes, which separates it strikingly from the related Andalusian species with $2n = 20(Xy_p)$ such as *T. (T.) inspersa* Rosenhauer, 1856, *T. (T.) marginicollis* Rosenhauer, 1856, *T. (T.) intermedia*, *T. (T.) lugens* Rosenhauer, 1856 and *T. (T.) carmelenae*, sharing a bifid mesosternum and elytra covered with sparse and fine puncturation.

Another species of *Timarcha* with $2n = 24$ chromosomes, *T. (T.) pratensis* (Duftschmid, 1825) (Petitpierre 1976), from Central and Eastern Europe, and Northern Italy, belongs to a very different group without any close interrelationship with *T. (T.) parvicollis* (Bechyné 1948, Warchalowski 2003).

Acknowledgements

I am very much indebted to Mr. Alain Grafteaux (Charleville-Mézières, France) for sending the sample of *T. metallica* and to Dr. José Antonio Jurado-Rivera (Palma de Mallorca, Spain), who helped me in collecting the specimen of *T. parvicollis* ssp. *seidlitzii* and by arranging and improving the micrographs.

References

- Angus R, Wilson CJ, Mann DJ A (2007) A chromosomal analysis of 15 species of Gymnopleurini, Scarabaeini and Coprini (Coleoptera: Scarabaeidae). Tijdschrift voor Entomologie 150: 201–211. <http://www.nev.nl/tve/pdf/te0150201.pdf>
- Bechyné J (1948) Contribution à la connaissance du genre *Timarcha* Latr. 12: Études phylogénétiques et zoogeographiques (Col. Phytophaga, Chrysomelidae). Sborník Národního Musea v Praze 4B(2): 1–62.
- Gómez-Zurita J (2008) Species and speciation in *Timarcha*. In: Jolivet P, Santiago-Blay JA, Schmitt M (Eds) New Developments in the Biology of Chrysomelidae, Brill, Leiden-Boston, 17–39.
- Gómez-Zurita J, Juan C, Petitpierre E (2000) The evolutionary history of the genus *Timarcha* (Coleoptera, Chrysomelidae) inferred from mitochondrial COII gene and partial 16S rDNA sequences. Molecular Phylogenetics and Evolution 14: 304–317. doi: 10.1006/mpev.1999.0712

- Gómez-Zurita J, Pons J, Petitpierre E (2004) The evolutionary origin of a novel karyotype in *Timarcha* (Coleoptera, Chrysomelidae) and general trends of chromosome evolution in the genus. *Journal of Zoological Systematics and Evolutionary Research* 42: 332–341. doi: 10.1111/j.1439-0469.2004.00267.x
- González-Megías A, Gómez JM (2001) Adult and larval plant range preference in *Timarcha lugens* (Coleoptera: Chrysomelidae): strict monophagy on an atypical host. *Annals of the Entomological Society of America* 94: 110–115. doi: 10.1603/0013-8746(2001)094[0110:AA LPRA]2.0.CO;2
- Iablokoff-Khnzorian SM (1966) Considérations sur l'édage des Chrysomelidae et son importance phylogénique. *L'Entomologiste* 22(6): 115–138.
- Jolivet P (1948) Contribution à l'étude des *Americanotimarcha* subgen.n. *Bulletin du Musée royal d'Histoire naturelle de Belgique* 24(43): 1–11.
- Jolivet P, Petitpierre E (1973) Plantes-hôtes connues des *Timarcha* Latreille (Col. Chrysomelidae). Quelques considérations sur les raisons possibles du trophisme. *Bulletin de la Société entomologique de France* 78: 9–25.
- Jolivet P, Petitpierre E (1992) Notes on *Timarcha*. *Chrysomela Newsletter* 26: 2.
- Jolivet P, Poinar GJr, Verma KK (2013) *Timarcha* Latreille: an strange beetle and a living fossil. *Terrestrial Arthropod Reviews* 7: 3–20. doi: 10.1163/18749836-06041071
- Kippenberg H (2010) Chrysomelidae. In: Löbl B, Smetana A (Eds) *Catalogue of Palaearctic Coleoptera vol 6 Chrysomeloidea*. Apollo Books, Stenstrup, Denmark, 390–443.
- Petitpierre E (1970) Cytotaxonomy and evolution of the genus *Timarcha* Latr. (Coleoptera: Chrysomelidae). *Genética Ibérica* 22: 67–120.
- Petitpierre E (1971) Contribuciones citogenéticas a la filogenia de las *Timarcha* (Col. Chrysomelidae). I Simposio Internacional de Zoofilogenia, Universidad de Salamanca, Salamanca 1969: 395–406.
- Petitpierre E (1976) Further cytotaxonomical and evolutionary studies on the genus *Timarcha* Latr. (Coleoptera: Chrysomelidae). *Genética Ibérica* 28: 57–81. doi: 10.1007/BF01937739
- Petitpierre E, Jolivet P (1976) Phylogenetic position of the American *Timarcha* Latr. (Coleoptera, Chrysomelidae) based on chromosomal data. *Experientia* 32: 157–158.
- Petitpierre E (1982) Chromosomal findings on 22 species of Chrysomelinae (Chrysomelidae: Coleoptera). *Chromosome Information Service* 32: 22–23.
- Petitpierre E (2011) Cytogenetics, cytotaxonomy and chromosomal evolution of Chrysomelinae revisited. *ZooKeys* 157: 67–79. doi: 10.3897/zookeys.157.1339
- Petitpierre E, Daccordi M (2013) Chrysomelidae (Coleoptera) de la sierras del Altiplano de Granada (Granada, Andalucía). *Zoologica Baetica* 24: 53–78. <http://www.ugr.es/~zool-bae/vol24/Zoo-4-Petitpierre color.pdf>
- Smith SG, Virkki N (1978) Coleoptera. In: John B (Ed.) *Animal Cytogenetics vol 3: Insecta* 5. Gebrüder Borntraeger, Berlin-Stuttgart, 366 pp.
- Warchalowski A (2003) Chrysomelidae. The leaf-beetles of Europe and the Mediterranean area. *Natura optima dux Foundation*, Warszawa, 600 pp.
- Warchalowski A (2010) The Palaearctic Chrysomelidae. Identification keys. Volume 1. *Natura optima dux Foundation*, Warszawa, 629 pp.